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Extreme Promiscuity in a Mating System Dominated by Sexual Conflict

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Coelopids live in wrack beds consisting of seaweed washed up on beaches. Their mating system is characterized by sexual conflict and convenience polyandry, with females resisting male mating attempts. We estimated the level of harassment by males and the success rate of rejection by females collected from a high density wild population. Males mounted a female every 8.41 min. Of these mounts 35% resulted in copulation. This suggests that females could be mated up to 5 times every 2 h. Females typically live for 3 weeks, and thus, could mate with hundreds of males during their lifetime. We found a 50:50 sex ratio throughout the wrack bed revealing that females do not avoid male harassment by leaving the wrack bed when not ovipositing.

KEY WORDS: sexual conflict; pre-mating struggle; convenience polyandry; sex ratio; male harassment; multiple mating.

INTRODUCTION

The mechanisms involved in sexual conflict have recently been a topic of much discussion (Chapman *et al*., 2003; Arnqvist and Rowe, 2005). One mating system characterized by overt sexual conflict is that of convenience polyandry (Thornhill and Alcock, 1983). Convenience polyandry occurs when males are able to coerce females into accepting unwanted copulations by increasing the likely costs of rejection beyond the costs of mating. There are a number of insect taxa in which this mating system is common,

including the gerrids (Rowe, 1992), the sepsids (Blanckenhorn *et al*., 2000; Muhlhauser and Blanckenhorn, 2004) and the coelopids (Day and Gilburn, 1997; Crean and Gilburn, 1998; Crean *et al*., 2000). One important factor determining the degree of convenience polyandry is population density, as this determines the extent of male harassment (Arnqvist, 1992).

In order to measure the degree of convenience polyandry present in a population, the number of lifetime matings of individual females needs to be estimated. However, there is a considerable lack of information regarding remating frequencies in insects displaying convenience polyandry.

The seaweed fly *Coelopa frigida* (Diptera: coelopidae) inhabits decomposing beach-cast wrack, known as wrack beds. Wrack beds form when detached seaweed is deposited onshore during storms or high tides. The adults live and breed within the wrack bed. Females oviposit on the seaweed and the larvae feed on the bacteria that decompose the seaweed and the sugars that are released during the decaying process (Cullen *et al*., 1987). Population densities vary considerably both temporally and spatially and can often be extremely high (Day and Gilburn, 1997). The mating system of *C. frigida* is characterized by a vigorous pre-mating struggle (Day and Gilburn, 1997). *C. frigida* has no elaborate courtship behaviour. Males simply attempt to mount females as soon as they are aware of them. Males are stimulated to mount females by the presence of seaweed (Dunn *et al*., 2002). Females, including virgins, invariably respond to being mounted by performing a rejection response, and a pre-mating struggle ensues. Seaweed does not affect female willingness to mate but does stimulate oviposition to a similar extent in both mated and virgin females (Dunn *et al*., 2002).

Female coelopids perform three behavioural responses to being mounted by a male: shaking, kicking and abdomen curling to avoid contact of genitalia. There are three possible outcomes from pre-mating struggles: (1) the male is successfully removed by the female rejection response; (2) the male withstands female rejection, the female becomes passive and the pair copulate; (3) the male resists the female rejection response, the female becomes passive but the male then dismounts without mating with the female. Males have been shown to preferentially dismount females with low egg loads (Pitafi *et al*., 1995) and females with low future survival prospects (Dunn *et al*., 2001).

Pre-mating struggles are indicative of a mating system based upon convenience polyandry (Thornhill and Alcock, 1983; Rowe *et al*., 1994). Large male coelopids gain a mating advantage in all coelopids so far studied (Crean *et al*., 2000) and this appears to occur as a result of their superior ability to withstand female rejection for longer (Crean and Gilburn, 1998). In other words, sexual selection favouring large male size occurs as a side effect of sexual conflict. The strength of sexual selection for large male size

varies considerably between populations (Day and Gilburn, 1997). However, female mate preferences are repeatable and unaffected by multiple interactions with males (Shuker and Day, 2001, 2002).

The rejection responses evolved by females to counteract male coercion are likely to be energetically costly to perform (Watson *et al.*, 1998). The main consequence of convenience polyandry is that females will allow themselves to be mated with, even if the mating is costly, when these costs are less than the likely costs associated with rejection. Though copulation is likely to have costs in *C. frigida*, females appear to copulate many more times than is necessary to fertilise their eggs. Males have remarkable stamina and many will continuously mount females if available, for example, one male *C. frigida* mounted 37 consecutive virgin females each within 5 min of the last and each female produced offspring (Gilburn, unpublished data). After numerous matings male willingness to mount does eventually drop, but not dramatically. This suggests that a very high level of convenience polyandry might occur in high density wild populations where males have effectively limitless opportunities to mate with females.

The initial aim of this study was to determine the operational sex ratio within a wrack bed to ascertain whether females actively avoid wrack beds when they are not ovipositing in order to evade harassment by males and reduce their number of superfluous matings. The second aim of this study was to determine how often males will mount and mate with females in a high density wild population, and from this, estimate the degree of convenience polyandry within this species.

METHODS

Determination of Sex Ratio in Wild Wrack Beds

This study was carried out at St Mary's Island (Tyne and Wear, North-East England, UK, ordnance survey grid ref: NZ349743) in summer 2002. Three contrasting study sites at least 150 m apart were chosen on the basis of the age of the wrack-bed present (determined by the stage of larval development of flies and the level of vegetative decay):

Site A was located in the upper zone of the shore in an area of sand. The seaweed present, which was comprised mostly of *Fucus* species, was relatively old—the upper layer of seaweed was extremely desiccated by weathering, and the layer underneath was well decomposed. The wrack bed was relatively shallow with a maximum depth of 12 cm. The majority of larvae present were in the final developmental instar stage. Dead larvae were also present among the desiccated seaweed.

Site B was located lower down the shore than Site A, at the upper edge of the tidal zone. The wrack bed was of an intermediate age and was comprised mostly of *Fucus* and *Laminaria* in roughly equal proportions. The lower layers of the wrack bed showed signs of decomposition but the seaweed in the upper levels was still intact with very little evidence of decomposition, though larvae at an intermediate stage of development were present. At the beginning of the study the maximum depth of the wrack bed was approximately 60 cm.

Site C showed a gradient from fresh seaweed in the intermediate tidal zone, which had been deposited only 2 or 3 days previously, to seaweed that had been present for 4 or 5 days in the upper tidal zone. The wrack bed was a mixture of *Fucus* and *Laminaria* species, with *Fucus* being the predominant seaweed present. The depth of the wrack bed ranged from approximately 20 cm in the intermediate tidal zone to approximately 50 cm in the upper tidal zone.

Sex ratios of *C. frigida* were determined using a revised version of the kick-sampling technique (Ausden, 1996), whereby a perspex cage was placed over the wrack bed and the seaweed underneath disturbed, causing the adult flies to fly up into the cage. The lid of the cage was then moved over the entrance, trapping the flies inside. The flies present were then aspirated into bottles according to sex. As several species of fly were present, any flies that were not *C. frigida* were discarded at this stage. Counts were made of both sexes. Ten collections were made from each site using a randomised block design.

Determination of Male Harassment and Mating Rates in the Wild

This part of the study was also carried out at St Mary's Island during the summer of 2003. During the study period population density was very high with searching males likely to encounter a female to mount within a matter of a few seconds. Coelopids mount, struggle and mate within the wrack bed, thus mating behaviour cannot be directly observed in the wild. However, coelopids will readily mate if placed together in Petri dishes. We collected wild flies from the wrack bed. Flies were collected using the kick-sampling technique as outlined for the sex ratio study. Male and female flies were aspirated into collection bottles and mating trials were carried out immediately after capture. The mating trials were carried out in an upturned Petri dish. Pairs of flies were placed into the Petri dish and the time taken for each male to mount the female was determined up to a maximum period of five minutes. The outcome of all mounts was scored either as: (1) a successful female rejection; (2) a copulation; or (3) a male dismount, if the male rejected the female after withstanding her rejection response. When

the male failed to mount a female within five minutes, the trial was scored as a non-mount. No fly was used in more than one trial. All trials were carried out in daylight.

We also carried out a similar experiment using virgin flies from the same population that were collected from the wild as larvae. The males were placed individually with minced seaweed for 24 h before use in the study. Females were maintained for the same period in groups of 30 with sugar solution as a food source. Mating trials were then carried out using the same procedure described above.

Assumptions

In order to estimate lifetime number of mounts and copulations we have made a number of assumptions. Firstly we have assumed that average female lifespan is 21 days. This is a conservative estimate of lifespan based upon previous studies of longevity (Burnet, 1960; Dobson, 1974; Collins, 1978), which revealed an average lifespan in the wild of 3–4 weeks.

Our estimate of male harassment rate is for day time only. Unlike other Dipterans such as *Drosophila*, which show clear bimodal morning and evening peaks in activity (Helfrich-Förster, 1998), *C. frigida* display a continuous level of activity throughout the day. Laboratory studies under red light have shown that males do continue to mount females during the night (Wood and Gilburn, unpublished results). However, because we did not estimated the mounting rate of wild males during the night time we have excluded night time behaviour from our estimate of the level of convenience polyandry. Thus the estimate of the extent of promiscuity within the study is likely to be conservative. We have assumed that mounting and mating occurs at the same rate for 12 h during the day.

The next assumption is that males come into contact with females with the same frequency in the mating chamber during the experiment, as they do within the wrack bed. The Whitley Bay site used was chosen because of its high population density. Population density within the wrack bed was so high that searching males were likely to be almost continuously meeting females. This is not untypical for this species.

RESULTS

Sex Ratio in Wrack Beds

It was found that the operational sex ratio (OSR) at site A did significantly deviate from 50:50, however it was females that were in excess

Table I. Total Number of Male and Female *C. frigida* Found at Each of the Three Test Sites (A, B and C), and *P*-Value for Deviation from a 50:50 Operational Sex Ratio Calculated by Binomial Tests

Site	Number of males	Number of females	*P*
A	235	283	0.039
B	891	936	0.303
C	416	423	0.836
Total	1542	1652	0.054

(Table I). No significant difference in OSR was found for sites B and C (Table I). A G-test comparing the OSR at the three sites reveals no significant differences ($G_2^2 = 2.45$, $P = 0.29$).

Mount and Mating Rates in the Wild

Of the 203 mating trials observed, 96 males attempted to mount their given within a total observation time of 678 min. This suggests that wild males will mount a female every 8.41 min. We found that females managed to successfully resist copulation in 38.5% of pre-mating struggles. The male itself terminated another 27.1% of mating attempts by dismounting the female after she had ceased resisting. The remaining 35.4% of male mating attempts resulted in a copulation. Assuming a constant rate of male mounting activity throughout the day, this suggests that females will mate over 30 times a day. Assuming a female lifespan of 21 days and a constant male mount rate, a female will be mounted approximately 1800 times in a lifetime. From this, we can assume that females may mate over 600 times in their lifetime.

A Comparison of the Mating Behaviour of Wild Caught and Virgin Flies

A total of 114 out of 118 virgin laboratory males mounted their given female within 5 min. This was a considerably higher level of male mounting ($\chi_1^2 = 98.2$, $P < 0.001$) than that exhibited by wild males (96 out of 203 mounted). Of the 96 wild males that mounted 26 dismounted their given female without copulation taking place. This was a much higher level of male dismounting ($\chi_1^2 = 145.1$, $P < 0.001$) than seen in virgin laboratory males (1 out of 114 dismounted). The proportion of females that successfully managed to reject their given male did not differ ($\chi_1^2 = 0.4$, $P < 0.53$) between wild collected (33 out of 96) and virgin laboratory flies (44 out of 114).

DISCUSSION

Here we reveal that female *C. frigida* that live in high density populations may be mounted by thousands of different males during their lifetime. This was estimated by measuring the rate at which males collected from a high density population will mount females. Even though males from this population have continual access to a large number of females, we found they will on average mount a female approximately every nine minutes when placed together within a Petri dish. This is lower than the rate exhibited by virgin males but is still remarkably high. We found that just over a third of male mating attempts result in a successful copulation. If males mount and female reject at the same rate in the Petri dishes as they do in the wild then females could be mating with as many as 30 different males every day, indicating extreme convenience polyandry within this species.

The finding that female *C. frigida* may be mounted and mated many hundreds of times during their lifetime suggests that there exists huge potential for sperm competition (Parker, 1970) and cryptic female choice to operate within this species (Thornhill, 1983; Eberhard, 1996). Studies using two males have revealed that both processes do operate in *C. frigida* (Blyth and Gilburn, 2005). Furthermore, as population density shows considerable temporal and spatial variation, *C. frigida* provides an excellent opportunity to investigate the effects of population density of post-copulatory sexual selection.

The comparison between the frequency of male dismounting in wild and laboratory-reared populations implies that there is a greater proportion of females that may be less desirable to males in the wild. It is unlikely to reflect a lower willingness to mate by males as unwilling males would not mount a female. Females in wild populations are likely to be less gravid than virgin females. Male mate choice for fecund females has previously been identified in *C. frigida* (Pitafi *et al.*, 1995) and was found to be an important factor in determining whether a male will dismount a female. Another potentially important factor is the female's age. Male mate choice for high female survivorship has been found in another coelopid, *Gluma musgravei* (Dunn *et al.*, 2001). It is likely that males will come into contact with a large proportion of older females in the wild, which they might determine as not being worth mating with, whereas in the laboratory, males are typically offered fully gravid females between 2 and 10 days old. The reason for this aversion to older females could be due to their shortened longevity and consequently, reduced future egg-laying potential. This is currently under investigation in *C. frigida*. The presence of a high level of male dismounting by wild males suggests that sperm or other ejaculate

components may be limited. This is particularly likely to be the case if males are mating with a new female approximately 30 times a day.

Females clearly do not leave wrack beds to avoid harassment by males even when they have already laid their egg clutches, indicating that there may be additional benefits in remaining close to the wrack bed that outweigh the costs of continuous male harassment. These benefits may include shelter, a source of nutrients and moisture, and 'safety in numbers.' It also suggests that the costs of sexual conflict are not particularly high to females. The fact that males are so willing to mate in this species even in a population where they have had continuous access to limitless females, suggests that the costs of conflict to males are very low.

Our results have shown that male harassment can result in extreme convenience polyandry, whereby females mate many hundreds of times more often than is required to ensure fertilisation of their eggs. Despite the extreme level of male harassment females did not avoid wrack beds and therefore avoid males. We are currently investigating the costs of harassment to both sexes and the potential benefits to females of remaining within a wrack bed.

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